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OXYGEN SCAVENGING SYSTEM

10/518292

This is a regular application filed under 35 U.S.C. §111(a) claiming priority under 35 U.S.C. §119(e)(1), of provisional application Serial No. 60/389,246 having a filing  
5 date of June 17, 2002 and filed under 35 U.S.C. §111(b).

**TECHNICAL FIELD**

The present invention relates generally to oxygen scavenging (i.e., targeting and reduction/elimination) for  
10 oxygen sensitive products, more particularly to a composition, system, and attendant methodology for removing oxygen from stored oxygen sensitive products such as food, pharmaceuticals, etc.

**BACKGROUND OF INVENTION**

The quality and character of products, whether they be consumables, intermediates, etc., remain of utmost importance. Furthermore, it is well known that freshness and shelf life can be key or determinative considerations in one's selection  
20 calculus. Although degradation is a natural phenomenon and can in fact be desirable, it more often than not is a condition sought to be controlled, more particularly eliminated, or at least slowed down, as is almost always the case with perishable goods.

Many substances, especially foods, benefit from storage in an environment free from, or containing a very low level of, free oxygen ( $O_2$ ). Oxygen is known to cause oxidative damage to many products, particularly, but not limited too, fats and oils. When exposed to oxygen many fats and oils oxidize, with a rancid flavor imparted to the fat or oil, other qualities and the general character of the oil being thereby altered. As the oxidation of fats and oils appears to be a self-catalytic reaction (i.e., upon initiation, the reaction proceeds relatively quickly, and fully), preventing or retarding the oxidation in the first place is paramount. Furthermore, and of equal importance, is the fact that oxygen supports the growth of microorganisms which cause spoilage and discoloration of the product.

Current packaging methods and packaging materials enable the elimination of much of the oxygen via time consuming vacuum and head space gas flushing processes. In most cases, some amount of oxygen remains in the package. Removal of oxygen from packages of products where gas is trapped within the product (e.g. bread or pasta) is especially difficult. Furthermore, most packaging materials are not impervious to oxygen penetration (i.e., package ingress: over time, oxygen leaks through the packaging material, and into the package).

To retard oxidation, anti-oxidants have been added to

foods. For instance, BHA [(1,1-dimethylethyl)-4-methoxy phenol] and BHT [2,6-di-tert-butyl-para-cresol] are common anti-oxidant food additives. However, BHA is regarded as moderately toxic by ingestion, and even though BHT is considered to have low toxicity, the use in foods of either of these compounds is limited. While these compounds have contributed greatly to the food industry by reducing the amount of food that must be discarded, some consumers prefer foods without them.

In a broader sense, the binding/scavenging of oxygen has typically been accomplished with iron oxide, more particularly, iron oxide packaged within gas permeable bags or sachets. Drawbacks associated with such approach have included, but are not limited to, careful sealed storage of the sealed package so as to prevent activation upon exposure to oxygen, the expense of such system, and the fact that iron oxide creates heat as it binds oxygen, a less than desirable outcome.

A further, widely practiced means of binding oxygen is the use of the enzyme glucose oxidase, in combination with a suitable glucose source, if necessary. Used alone, glucose oxidase creates or generates peroxide (i.e., hydrogen peroxide is a reaction product). Peroxide may have detrimental effects on the product in the package and its presence may limit the

further binding of oxygen by the glucose oxidase. The addition of an appropriate amount of catalase enzyme has been used to break down the peroxide. This works acceptably in many systems where the glucose oxidase/catalase mixture is spread  
5 over a surface, and the packaged product acts as a pH buffer, maintaining an acceptable pH range where the oxygen binding reaction proceeds, however, efforts to use such enzymatic formulations in a dispersed or contained form on iron rich products have been unsuccessful because of discoloration of  
10 the product.

It is believed that the lack of success with such enzymatic formulations is most likely due to the oxygen binding reaction being self-limited by the change in pH within the bag or sachet (see generally Enzyme Technology, Chaplin &  
15 Bucke, Cambridge University Press, 1990). As enzymes are amphoteric molecules containing a large number of acid and basic groups, mainly situated on their surface, the charges on these groups will vary, according to their acid dissociation constants, with the pH of their environment. In addition to  
20 the reactivity of the catalytically active groups, this directly impacts the total net charge of the enzymes, and the distribution of charge on their exterior surfaces. These effects are especially important in the vicinity of the active sites. Thus, in combination, the variability of the charges

with pH (i.e., the charges being a function of pH) affect the activity, structural stability and solubility of the enzyme, and have thus been limitations upon this form of oxygen scavenging system.

5           Although the use of a buffer to stabilize a liquid glucose oxidase system during storage is described in European patent EP0418940, it does not address oxygen removal or buffering of the system during enzyme activity. Similarly, U.S. Pat. Nos. 2,765,233 and 5,064,698 teach the addition to  
10           glucose oxidase directly to packaging materials in various ways, namely via encapsulation in polymeric beads. Be this as it may, there are practical limitations on the amount of glucose oxidase that can be applied to food wrappers/containers utilizing heretofore known techniques. No  
15           commercially acceptable oxygen scavenging agent, suitable for direct application or subsequently introduced post packaging, which virtually eliminates (i.e., binds) oxygen present and which further insignificantly changes or modifies the quality or character of the perishable (e.g., does not discolor the  
20           item), has been heretofore disclosed.

#### SUMMARY OF THE INVENTION

The subject oxygen scavenging system is generally directed to the elimination of oxygen from packaged oxygen

sensitive products where moisture is present or may become present. An oxygen scavenging composition of the subject invention includes an enzyme system (e.g., an oxidoreductase enzyme), a suitable energy source for the enzyme system, and  
5 a buffer. The composition, which enhances the shelf-life of a packaged product, is suitable for direct application to the product of the packaged product with no consumer detectable change in product character. The composition binds oxygen when exposed to moisture, thereby reducing the level of oxygen in  
10 a closed (e.g., sealed) space such as a food package or the like. The system of the subject invention contemplates the scavenging composition in combination with a discrete water permeable "housing" within which the composition is contained, or as an integral element or component of a perishable storage  
15 container or the like. More specific features and advantages will become apparent with reference to the DETAILED DESCRIPTION OF THE INVENTION, and appended claims.

#### DETAILED DESCRIPTION OF THE INVENTION

20 The oxygen scavenging system of the subject invention includes a composition comprising an enzyme system (e.g., an oxidoreductase enzyme), a suitable energy source or substrate for the enzyme system, and a buffer, the composition scavenging or binding oxygen when exposed to moisture, thereby

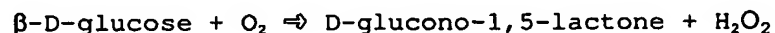
reducing the level of oxygen in a closed (e.g., sealed) space such as a food package or the like. Preferably, the enzyme system includes an oxidoreductase enzyme, more particularly a dry glucose oxidase, and the energy source comprises a reducing sugar, more particularly, a glucose source. The composition of the subject invention preferably further includes an effective amount of catalase. For example, the composition includes glucose oxidase in an amount of between 1 and 100 activity units (U) per gram, catalase in an amount of between 1 and 300 activity units (U) per gram, a glucose source in an amount of between about 20 and 99 percent by weight, and a buffer in an amount of between about 1 and 80 percent by weight. Further still, and preferably, the glucose source is dextrose, with the buffer preferably comprising sodium bicarbonate.

As will later be discussed, in addition to the composition of the subject invention, an oxygen scavenging system is disclosed wherein the system includes a water permeable enclosure (e.g., a bag, sachet, laminated sheet, or other three dimensional form) for housing or containing the subject composition. Furthermore, in lieu of containment, encapsulation, or general integration with storage media, the composition of the subject invention may be formed into solid or semi-solid three dimensional forms in addition to being

directly introduced in powder form to, or with, packaged oxygen sensitive products.

Oxidoreductases are enzymes which catalyze oxidation or reduction reactions (i.e., reactions in which hydrogen or oxygen atoms or electrons are transferred between molecules). This extensive class includes the dehydrogenases (hydride transfer), oxidases (electron transfer to molecular oxygen), oxygenases (oxygen transfer from molecular oxygen) and peroxidases (electron transfer to peroxide). For example: glucose oxidase (EC 1.1.3.4, systematic name,  $\beta$ -D-glucose: O<sub>2</sub> 1-oxidoreductase), or, hexose oxidase (EC 1.1.3.5, systematic name, D-hexose: O<sub>2</sub> 2-oxidoreductase,), each of which will be subsequently discussed.

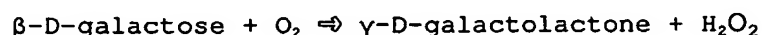
Glucose oxidase is a highly specific enzyme for D-glucose, from the fungi *Aspergillus niger* and *Penicillium*, which catalyses the oxidation of  $\beta$ -D-glucose to D-glucono-1,5-lactone, which spontaneously hydrolyzes, non-enzymically, to gluconic acid, using molecular oxygen, with a release of hydrogen peroxide as follows:



Hexose oxidase, which also functions as an effective oxygen scavenger and is less specific than glucose oxidase, is an enzyme which in the presence of oxygen is capable of oxidizing D-glucose, and several other reducing sugars (i.e.,



substrates) including, but not limited to maltose, lactose and cellobiose, to their corresponding lactones, with subsequent hydrolysis to the respective aldobionic acids (e.g., in the case of D-glucose, gluconic acid). Accordingly, hexose oxidase  
5 differs from another oxidoreductases (e.g., glucose oxidase) which can only convert D-glucose in that this enzyme can utilize a broader range of sugar substrates. Hexose oxidase oxidation catalysis may be illustrated, for example for glucose and galactose, as follows:

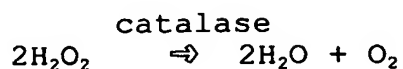


The capability of oxygen oxidoreductases, such as glucose oxidase and hexose oxidase, to generate hydrogen peroxide, which has an antimicrobial effect, has been utilized to  
15 improve the storage stability of certain food products including cheese, butter and fruit juice as it is disclosed in JP-B-73/016612. It has also been suggested that oxidoreductases may be potentially useful as oxygen scavengers or antioxidants in food products.

20 As noted with respect to the above enzymatically catalyzed oxidation reactions, hydrogen peroxide is characteristically a reaction product thereof (i.e., most metabolism in the presence of atmospheric oxygen leads to the production of hydrogen peroxide). It is known to catalytically

decompose hydrogen peroxide, and thus eliminate the toxic bactericidal effects thereof, to form water and molecular oxygen, utilizing the enzyme catalase which is derived from the same fungal fermentations as glucose oxidase, as follows:

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For most large-scale applications the two enzymic activities are typically not separated (i.e., oxygen oxidase and catalase may be used together when net hydrogen peroxide production is to be avoided, and thus inhibition of the oxygen scavenging process by the peroxide).

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As to enzymatic activity, it is widely known that the amount of enzyme present or used in a process is difficult to determine in absolute terms (i.e., mass), as there is an inherent variability in purity, with arguable more relevant parameters being the activity of the enzyme preparation and any contaminating enzymes. A widely known unit of enzyme activity (i.e., an "activity unit," U), adopted in 1964 by the International Union of Biochemistry (International Union of Biochemistry. *Enzyme Nomenclature: Recommendations 1964 of the International Union of Biochemistry*. Amsterdam: Elsevier, 1965.), is the amount of enzyme activity which will catalyze the transformation of 1 micromole of the substrate per minute

under standard conditions. Typically, this represents about 10EE-6 to 10EE-11 kilograms for pure enzymes, and about 10EE-4 to 10EE-7 for industrial enzyme preparations. Another unit of enzyme activity has been recommended, namely, the katal (kat) which is defined as the amount which will catalyze the transformation of one mole of substrate per second (1 kat = 60,000,000 U). Furthermore, non-standard activity units are used, such as Soxhet, Anson and Kilo Novo units, which are based on physical changes such as lowering viscosity and supposedly better understood by industry.

Suitable energy sources or substrates for the composition of the subject invention include carbohydrates (i.e., saccharides), more particularly reducing sugars (i.e., those capable of reducing a mild oxidizing agent, such as Fehling's reagent). Early biochemists devised analytical methods for the detection and quantification of saccharides. One of these test, Fehling's reagent, was based upon the aldehyde (RCOH) or ketone (RCOR) groups present in the saccharide structures: the reagent oxidized the saccharide while the saccharide reduced the oxidation state of the ions of the reagent. Generally, saccharides form rings that involve the aldehyde or ketone group. Reversible ring formation is possible unless the hemiacetal or ketal hydroxyl group has become involved with another link. Rings that are locked have no aldehyde or ketone

group to react, unless there are several rings and at least one can open, and are referred to as non-reducing sugars.

Saccharides may be generally classified as mono-, di-, oligo-, or poly- saccharides, the fundamental feature being the ability to be either decomposed by hydrolysis, as is the case with the di- and polysaccharides, or not be decomposed by hydrolysis, as is the case with monosaccharides. Another feature of saccharides, more particularly monosaccharides, is that because of their hydroxyl groups (-OH), rings can be joined together, as by intermolecular dehydration, to form disaccharides (e.g., a union of  $\alpha$ -D-glucose molecules forms maltose). The disaccharides, in turn, may be further dehydrated to join more rings together and form polysaccharides (e.g., starting again with  $\alpha$ -D-glucose, starch and glycogen may be formed therewith, while starting with  $\beta$ -D-glucose, cellulose may be formed: the enzymes that hydrolyze  $\beta$  linkages in cellulose are different from those that hydrolyze  $\alpha$  linkages). Typically, in their capacity as reducing sugars, disaccharides reduce half as quickly and half as much as an equal weight of other similar monosaccharides (e.g., maltose/glucose).

Preferable reducing sugars for inclusion in the subject composition include, but are not limited to the monosaccharides glucose, galactose, fructose, xylose,

arabinose, mannose, rhamnose; the disaccharides maltose, isomaltose, lactose, cellobiose; and, starches (i.e., polysaccharides) such as amylose and amylopectin.

As previously noted, in addition to the composition of the subject invention being placed directly into a package either in contact with, or separate from, the packaged product, bags, sachets, or other forms containing the composition of the subject invention can be produced and placed within packages of any moist product where it will consume oxygen within or entering the package. After the package is sealed and moisture contacts the composition of the subject invention, the oxygen level within the package will decrease and will be maintained at a very low level. The quantity of the composition required in a package to achieve and maintain a very low level of oxygen is a function of the amount of oxygen present in the package when it is sealed, in addition to the quantity of oxygen expected to penetrate the package during the life of the package. For convenience, the composition may be placed in a variety of containers such as bags, sachets, laminated sheets, and numerous three dimensional forms. The container or enclosure for the composition of the subject invention needs to be water permeable. It is further noted and contemplated that the composition of the subject invention may also be compressed or

otherwise formed into a solid or semi-solid three dimensional shape for direct placement into packages. Although the oxygen scavenging system of the subject invention contemplates various mechanisms by which the composition effectively "shares" a head or similar space with the packaged product, the critical consideration is that the composition of the subject invention be placed within or be otherwise integrated so as to be within the package such that it is exposed to moisture.

As to a representative formulation for the composition, namely, one comprising glucose oxidase, dextrose, catalase and a buffering agent (e.g., sodium bicarbonate), for every mole of dextrose and oxygen acted on by glucose oxidase, one mole of both lactic acid and hydrogen peroxide are produced. Catalase acts on the hydrogen peroxide to produce one mole of water and one half mole of oxygen. The buffering or pH neutralizing agent dampens or counteract the pH reduction caused by formation of the acid. The elimination or mitigation of acid inhibition of the enzymatic process is especially advantageous for thorough oxygen scavenging. Several different neutralizing agents may be used, but the preferred buffering agent is sodium bicarbonate because of its pH buffering capacity, the large carbon dioxide release, and food grade status. The molar ratio between the glucose and buffering

agent can vary between from about 0.5 to 1 and 10 to 1, but preferably is 2 to 1.

Initial bench scale tests were conducted by blending one part sodium bicarbonate to two parts of OxyVac™ (Nutricepts, Inc., Burnsville, MN 55337) to balance the moles of dextrose and sodium bicarbonate. For the glucose oxidase and the catalase (Amano Enzyme USA Co., Ltd. Elgin, IL), one activity unit is defined as the quantity of enzyme that will oxidize 1 micromole of glucose or hydrogen peroxide, respectively, per minute utilizing the prescribed vendor assay method. The test composition of the subject invention is characterized as follows:

Constituents	Effective Amounts	Mass (grams)
glucose oxidase	3,300 units	
catalase	3,300 units	
dextrose		65.5
sodium bicarbonate		34
		Total: 100

Five grams of the aforementioned mixture or composition was introduced into an emptied tea bag and placed in a heat sealed poly food storage bag containing a moistened paper towel. After approximately 24 hours, the oxygen level in the bag was measured at 0.3 percent utilizing standard oxygen sensing/indicating apparatus (e.g., Quantek O<sub>2</sub> analyzer). At

approximately 48 hours, the oxygen level measured 0.0 percent.

Thereafter, thirty, 5 gram (g) sachets of the aforementioned composition were fabricated using coffee filter material. The filled fiber envelopes were subsequently placed in heat sealed poly bags with a variety of oxygen sensitive products. The following table summarizes head space oxygen levels (vol%,) as a function of time, for the listed items:

Item	Temperature	O <sub>2</sub> Level		
		0	24 hours	6 days
Water added Ham	40°F	20%	1.5%	0.0%
Summer Sausage	70°F	20%	4.3%	0.0%
Sliced Roast Turkey	40°F	20%	0.2%	0.0%
Fresh packaged Tortellini	40°F	20%	4.3%	0.1%
Shredded Potatoes	40°F	20%	0.2%	0.0%
Beef Sausage	40°F	20%	0.1%	0.1%
Damp Paper Towel	70°F	20%	0.0%	0.0%

No observable color changes were detected during the aforementioned tests.

In a second test set, the composition of the subject invention was placed in direct contact with several of the items previously listed and placed in heat sealed poly bags.

The following results are provided:



Item	Temperature	O <sub>2</sub> Level		
		0	24 hours	6days
Water added Ham	40°F	20%	0.0%	0.0%
Summer Sausage	70°F	20%	0.8%	0.0%
Sliced Roast Turkey	40°F	20%	0.0%	0.0%

A slight color change was noted for the summer sausage after six days.

It will be understood that this disclosure, in many respects, is only illustrative. Changes may be made in details, particularly in matters of shape, size, material, and arrangement of parts without exceeding the scope of the invention. Accordingly, the scope of the invention is as defined in the language of the appended claims.